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Biochemical regulation of rubber biosynthesis in guayule (*Parthenium argentatum* Gray)

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Abstract

Natural rubber is an irreplaceable raw material vital to industry, transportation, medicine and defense. It is largely produced from clonal plantations of *Hevea brasiliensis* in southeastern Asia. Temperate-zone rubber-producing crops are greatly desired to increase biodiversity, protect supplies, and provide a safe natural-rubber alternative for the large numbers of people suffering from Type I latex allergy to proteins in latex products. *Parthenium argentatum* (guayule) is currently under development in the United States as a suitable source of hypoallergenic latex. Improved *P. argentatum* lines are still desired with higher latex yields, improved agronomic characteristics, and broader growth range. Understanding the biochemical regulation of rubber yield (principally rate of synthesis) and quality (principally molecular weight distribution) in *P. argentatum* is an essential preliminary to the identification and manipulation of key regulatory steps. In this paper, the biochemical regulation of rubber biosynthesis in *P. argentatum* will be discussed and unique features highlighted. We find that the *P. argentatum* rubber transferase has kinetic features, such as high K_m^{IPP}, similar to other rubber producing species. However, *P. argentatum* also has some unique rubber transferase features, including very low K_m^{FPP} and negative cooperativity for C_{15} farnesyl pyrophosphate (FPP), which affect the way guayule regulates rubber biosynthesis.

Keywords: Allylic pyrophosphate; Guayule; Isopentenyl pyrophosphate; Molecular weight

1. Introduction

The United States is the world's largest single rubber consumer, using approximately 20% of the global supply of 6.9 million metric tonnes for its commercial, medical, transportation, and defense industries. It imports about 1.2 million metric tonnes of raw natural rubber for manufacturing at a cost approaching $2 billion, in addition to its enormous finished goods imports (over $8 billion, containing 350,000 metric tonnes of rubber). In addition, in 2002, the three principal rubber-producing countries—Thailand, Malaysia, and Indonesia, formed a cartel-like rubber triumvirate. The immediate goal of the triumvirate is to reduce rubber production by 4%, exports by 10%, and drive-up natural

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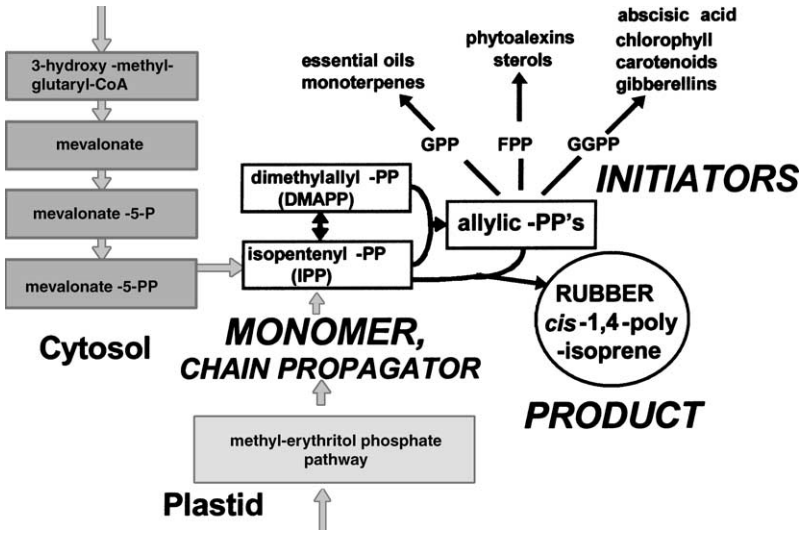

Fig. 1. A section of the isoprenoid pathway illustrating the branch to rubber biosynthesis. Rubber (the product) is synthesized in the cytosol from one molecule of allylic pyrophosphate (the initiator) and many molecules of isopentenyl pyrophosphate (the monomer). Isopentenyl pyrophosphate is produced by the cytosolic mevalonate pathway and by the plastidic methyl-erythritol pathway, as indicated by the shaded boxes.

rubber prices. Unlike fossil fuel, the United States has no domestic source of natural rubber to ameliorate the effects of the triumvirate and prices are expected to increase substantially for several years to come.

Parthenium argentatum Gray (guayule) produces high quality rubber in its bark parenchyma (Bonner, 1943; Backhaus, 1985; Madhavan et al., 1989; Whitworth and Whitehead, 1991) and is currently under commercial development initially as a source of hypoallergenic latex (Siler and Cornish, 1994; Siler et al., 1996; Cornish, 1996, 1998; Nakayama et al., 1996; Cornish et al., 2001). In order to further increase yields in guayule, the regulation of rubber yield and quality must be understood at the biochemical and genetic levels. Subsequent metabolic engineering can then be focused and targeted to increase rubber yield (primarily biosynthetic rate) and quality (primarily molecular weight) while minimizing or avoiding detrimental effects to the plant itself.

Natural rubber is a secondary product, compartmentalized in cytosolic rubber particles, that is not recatabolized during the life of the plant. Although soluble rubber transferases have been reported (Archer et al., 1963, and see literature reviews in Cornish, 1993 and Cornish and Siler, 1996), rubber polymerization is catalyzed by a rubber particle-bound enzyme (Archer and Audley, 1987; Berndt, 1963; Lynen, 1969; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Cornish and Siler, 1996); the earlier reports can be explained by the role of the soluble enzymes in the synthesis of allylic pyrophosphate initiator molecules. The membrane-bound rubber transferase (*cis*-prenyl transferase, EC 2.5.1.20) is associated with the rubber particles (Archer et al., 1963; Berndt, 1963; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Cornish et al., 1993; Cornish and Siler, 1996). Rubber transferase produces the rubber polymer (*cis*-1,4-polyisoprene) from isoprene monomers, which it links, chain-like, to an allylic pyrophosphate initiator molecule. The rubber polymer grows as more isoprene units are added to the chain. The rubber polymers are packaged inside sub-cellular, membrane-bound rubber particles (Siler et al., 1997; Cornish et al., 1999; Wood and Cornish, 2000). Each rubber transferase is capable of producing many rubber polymers in series, but the eventual polymer molecular weight varies considerably among species (Cornish and Siler, 1995; Castillón and Cornish, 1999; Cornish et al., 2000). The substrates for rubber biosynthesis, isopentenyl pyrophosphate (IPP) (the monomer), and its allylic pyrophosphate (allylic-PP) catabolites (the initiators), are synthesized from carbohydrates via acetyl-coenzyme

A, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and mevalonate (Fig. 1) (Chappell, 1995). The plastid-localized deoxy-xylulose/methyl-erythritol phosphate pathway also produces IPP (Rohmer et al., 1993; Eisenreich et al., 1998), but it is not yet known if this contributes to the cytosolic IPP pool from which the rubber polymers are synthesized.

In addition, rubber biosynthesis in *P. argentatum* is under strong environmental control, the rubber transferase being induced at the onset of winter (Bonner, 1943; Goss et al., 1984; Downes and Tonnet, 1985; Gilliland and van Staden, 1986; Madhavan et al., 1989; Appleton and van Staden, 1989, 1991; Ji et al., 1993; Sundar and Ramachandra Reddy, 2000, 2001; Cornish and Backhaus, 2003), but we shall not further cover this environmental aspect of rubber biochemistry in this report on the biochemical regulation of rubber biosynthesis in *P. argentatum*.

2. Materials and methods

2.1. Materials

Mature, field-grown *P. argentatum* (line 11591) plants were grown at the USDA, ARS, U.S. Water Conservation Laboratory, Phoenix, AZ. Branches were harvested, dipped into 1% aqueous ascorbate, sealed in plastic and shipped overnight on ice to Albany, California. *Ficus elastica* plants were purchased from a local nursery and grown in a greenhouse in Albany, California. Latex was tapped from the stems and petioles and collected in iced collection buffer. *Hevea brasiliensis* (line PB260) latex was donated by the Rubber Research Institute of India. Unlabelled IPP, DMAPP, GPP, all *trans*-FPP and all *trans*-GGPP as well as [^{14}C]IPP (55 mCi/mM) were obtained from American Radiolabeled Chemical Inc., St. Louis, MO.

Siliconized 1.5 ml tubes were supplied by USA Scientific (Ocala, FL, USA). Ready Safe scintillation fluid was purchased from Beckman Instruments (Fullerton, CA, USA). Chemicals, unless otherwise noted were purchased from Sigma (St. Louis, MO, USA).

2.2. Preparation of washed rubber particles

Enzymatically-active washed rubber particles were purified as described from *P. argentatum* (Cornish and Backhaus, 1990), *H. brasiliensis* (Cornish, 1993) *F. elastica* (Cornish and Siler, 1996). All particles were stored as glycerol-stabilized beads in liquid nitrogen until used (Cornish and Bartlett, 1997).

2.3. Rubber biosynthesis assays

IPP-incorporation rates were assayed in washed rubber particles using methods previously described for *P. argentatum* (Cornish and Backhaus, 1990), *H. brasiliensis* (Cornish, 1993), and *F. elastica* (Cornish and Siler, 1996). The reaction was begun by the addition of washed rubber particles to 50 μl of reaction mixture in 1.7 ml microfuge tubes and then floating the tubes in a temperature controlled circulating water bath. After 4 h at 25 °C for *H. brasiliensis* and *F. elastica*, and at 16 °C for *P. argentatum*, the reaction was stopped by the addition of 2.5 μl 500 mM EDTA, and the particles harvested by filtration. Filters were oven-dried at 37 °C overnight, washed individually with 5 ml of 1 M HCl and 3 × 4 ml 95% ethanol, and placed individually into vials with 5 ml of scintillation fluid. The amount of [^{14}C]IPP incorporation was determined by liquid scintillation counting using Beckman LS6500 (Beckman Coulter, Fullerton, CA, USA).

3. Results and discussion

3.1. Biosynthetic rate

In all rubber-producing species so far investigated, rubber transferase requires an allylic pyrophosphate (allylic-PP) substrate (produced by soluble *trans* prenyl transferases) to initiate polymer formation, and a divalent cation, such as Mg^{2+} or Mn^{2+}, as cofactor (Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Tanaka et al., 1996; Scott et al., 2003). The initiator normally used in vivo by rubber transferase appears to be the C_{15} farnesyl pyrophosphate (FPP) (Tanaka et al., 1996), although rubber transferases are able to accept a wide range of allylic-PP's as initiating substrate (Berndt, 1963; Lynen, 1969; Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Cornish and Siler, 1995; Cornish et al., 1999), and Mg^{2+} is the in vivo cofactor (Scott et al., 2003).

Enzymological investigations ideally are performed using a soluble enzyme system where

Enzyme + Substrate → EnzymeSubstrate complex → Enzyme + Product. This equation does not adequately describe rubber biosynthesis, which involves a membrane-bound enzyme, two substrates, a cofactor and a polymeric product that is not fully released from the active site at each substrate addition. We can describe this system as:

$$\begin{aligned}&\text{Enzyme} + \text{Substrate}_1\\ &\quad\rightleftharpoons \text{EnzymeSubstrate}_1 + \text{Substrate}_2\\ &\quad\rightarrow \text{EnzymeSubstrate}_2\text{Substrate}_1 + (\text{Substrate}_2)_n\\ &\quad\rightarrow \text{Enzyme}(\text{Substrate}_2)_{n+1}\text{Substrate}_1\\ &\quad\rightarrow \text{Enzyme} + \text{Product}\end{aligned}$$

where Substrate_1 is the allylic-PP initiator, Substrate_2 is the isopentenyl monomer, and Product ($(\text{Substrate}_2)_n\text{Substrate}_1$) is the *cis* 1-4 polyisoprene (rubber). Soluble enzyme activity has only once been reported for *P. argentatum* rubber transferase (Madhavan and Benedict, 1984), but most studies have relied upon the intact rubber particle membrane-bound system. Membrane-bound enzymes can be investigated intact provided that only one enzyme is present that uses the substrates for the reaction under investigation. This, fortunately, has proved to be the case for rubber biosynthesis (Cornish and Backhaus, 1990; Cornish, 1993; Cornish and Siler, 1996).

Rubber biosynthesis is an unusual reaction in that the rubber transferase can accept any one of a number of allylic-PPs as the initiating molecule (Berndt, 1963; Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Cornish and Siler, 1995; Cornish et al., 1999). However, the structure and size of the initiating molecule affect the IPP condensation reaction rate as the rubber molecule polymerizes (Fig. 2). In non-limiting allylic-PP concentrations, the longer the carbon chain of the initiator, up to the C_{15} farnesyl pyrophosphate (FPP), the higher the rate (v) of IPP incorporation into rubber by the *P. argentatum* rubber transferase. In this species, the C_{20} initiator, geranylgeranyl pyrophosphate (GGPP) leads to a lower IPP polymerization rate with only the C_5 dimethyl allyl pyrophosphate (DMAPP) giving a slower rate (Fig. 2).

When the kinetics of these reactions were analyzed further using plots of v against v/[S]

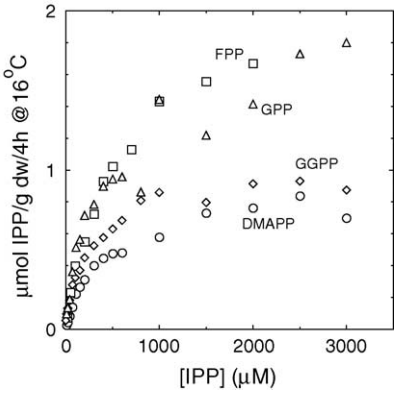

Fig. 2. Dependence of IPP incorporation rate by rubber transferase on IPP concentration in 20 μM allylic pyrophosphate initiator: (◯) DMAPP; (△) GPP; (□) FPP; (◇) GGPP.

(Woolf–Augustinsson–Hofstee plot (Segel, 1993)), the main part of the plots was approximately linear confirming the presence of a single IPP binding enzyme on the *P. argentatum* rubber particle (Fig. 3). The inverse of the gradient of such plots reflects the binding constant (apparent K_m) or the affinity of the enzyme for the substrate: the larger the K_m, the lower the affinity. In this case, the K_m^{IPP} is similar in all four initiators. Additional analysis using Hill plots (Fig. 4) also revealed that IPP incorporation rates, in non-limiting 20 μM APP, approximated Michaelis–Menten kinetics for single enzymes (the dashed line), and allowed a clearer determination of K_m^{IPP} ($x = K_m^{IPP}$ where $y = 1$). Although differences in K_m^{IPP} were apparent (Table 1),

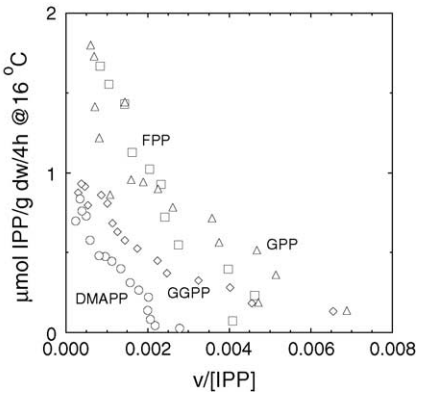

Fig. 3. Woolf–Augustinsson–Hofstee plot of IPP incorporation rate as a function of rate divided by IPP concentration in 20 μM allylic pyrophosphate initiator: (◯) DMAPP; (△) GPP; (□) FPP; (◇) GGPP.

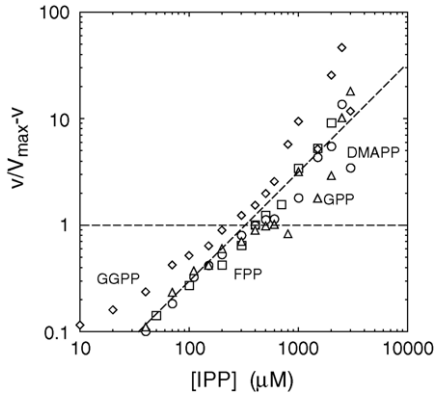

Fig. 4. Hill plot of IPP incorporation rate as a function of IPP concentration in the presence of 20 μM allylic pyrophosphate initiator: (◯) DMAPP; (△) GPP; (□) FPP; (◊) GGPP. The diagonal dashed line indicates a gradient of 1. The intercept of the data lines with the horizontal dashed line indicates K_m^{IPP}.

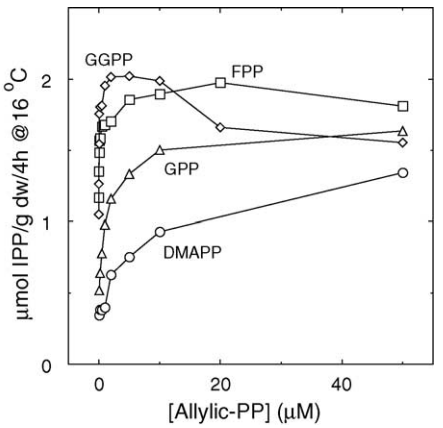

Fig. 5. Dependence of IPP incorporation rate on allylic pyrophosphate initiator concentration in 1 mM IPP: (◯) DMAPP; (△) GPP; (□) FPP; (◊) GGPP.

all values are high, as has been reported previously for IPP in the presence of FPP (Cornish et al., 2000; Cornish, 2001a,b). However, it is noteworthy that the lower IPP incorporation rate in the presence of GGPP as the initiator (Fig. 2) is not due to a decrease in the affinity of the rubber transferase for IPP compared with the affinity in the presence of GPP or FPP. In fact, the IPP affinity is actually greatest in GGPP (Fig. 4, Table 1).

Rubber biosynthetic rates also were investigated with varying amounts of allylic-PP in 1 mM IPP, and again were dependent upon allylic-PP concentration and identity (Fig. 5). At concentrations up to 10 μM allylic-PP, IPP incorporation rates increased with the size of the initiator. At higher concentrations, the rate in GGPP declined to below that in FPP and GPP, in agreement with the IPP concentration dependence seen earlier (Fig. 2). However, when the v against v/[S] analysis was performed (Fig. 6), it became apparent that the plots in the shorter initiators were far from linear, and the depth of the curve increased as the initiator shortened from FPP to GPP to DMAPP. These curves indicate that multiple binding constants are involved in the reaction, and led to the interpretation that the rubber transferase active site contains a non-specific hydrophobic binding region that traverses the particle monolayer membrane and with which the growing rubber molecule interacts until the rubber core in reached (Cornish, 2000, 2001a,b). Because DMAPP is an ef-

Table 1
Substrate binding constants for IPP in the presence of different allylic-PP initiating substrates and the different allylic-PPs

Initiator	Size (number of carbons)	K_m^{IPP} (μM)	K_m^{APP} (μM)
DMAPP	5	400	3.52
GPP	10	400	0.50
FPP	15	370	0.02
GGPP	20	240	0.02

Apparent K_m's were calculated from log plots of $v/(V_{max}-v)$ against [S]. Experimental details in Section 2. Reactions were performed for IPP in the presence of 20 μM allylic-PP and for the allylic-PPs in the presence of 1 mM IPP.

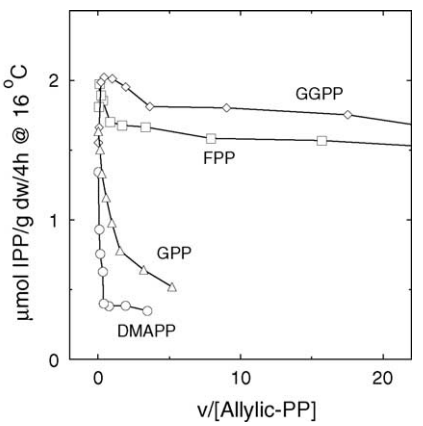

Fig. 6. Woolf–Augustinsson–Hofstee plot of IPP incorporation rate as a function of rate divided by allylic pyrophosphate initiator concentration in 1 mM IPP: (◯) DMAPP; (△) GPP; (□) FPP; (◊) GGPP.

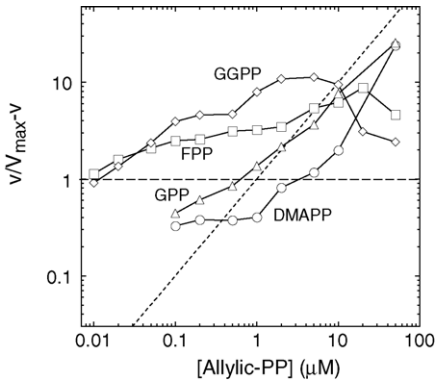

Fig. 7. Hill plot of IPP incorporation rate as a function of allylic pyrophosphate initiator concentration in 1 mM IPP: (◯) DMAPP; (△) GPP; (□) FPP; (◇) GGPP. The diagonal dashed line indicates a gradient of 1. The intercept of the data lines with the horizontal dashed line indicates K_m^{IPP}.

fective initiator of rubber biosynthesis, the specific allylic pyrophosphate binding site probably recognizes the allylic carbon and pyrophosphate and specifically binds via an amino acid/magnesium ion/pyrophosphate bridge in a manner comparable with other prenyl transferases (aspartate being the amino acid employed (Chen et al., 1994; Tarshis et al., 1994; Chang et al., 2003)). The rubber transferase presumably binds the same small portion of the larger allylic pyrophosphate molecules. A non-specific hydrophobic binding region may account for the higher binding affinity of the larger allylic pyrophosphate initiators and the greater this hydrophobic interaction between the allylic pyrophosphate and the binding site, the higher the overall affinity of the enzyme for the substrate.

Hill plots of the allylic-PP concentration dependent IPP incorporation (Fig. 7) indicate that, unlike IPP, the binding affinity of the rubber transferase for the different allylic-PPs varies considerably. The K_m^{FPP} and K_m^{GGPP} are much lower than the K_m^{GPP} or K_m^{DMAPP} (Fig. 7, Table 1). It is also apparent that, unlike IPP, FPP and GGPP generate Hill plots with gradients of substantially less than one, indicating strong negative cooperativity in the *P. argentatum* rubber transferase for these two substrates. Thus, when a new rubber molecule is initiated by FPP (or GGPP), it becomes much more difficult for another initiator to bind. This directly inhibits the chain-transfer reaction and allows the rubber molecule to continue to grow. This property of the *P. argentatum* rubber transferase may be the reason why the rubber produced during the summertime still appears to be of high molecular weight, even though the overall rate of synthesis is slow. Of course, in vivo it is unlikely that the plastid-produced GGPP initiates rubber biosynthesis, whereas FPP is produced in the cytosol and shares this compartment with the rubber particle-bound rubber transferase. The low affinity of the rubber transferase for DMAPP and GPP suggests that these substrates play little role in initiation in vivo regardless of their relative cytosolic and plastidic prevelance. Plant GGPP synthases are expressed in plant plastids and expression studies in latex indicate intra-organellar GGPP synthesis in non-chloroplastidic organelles such as lutoids and Frey-Wyssling particles and mitochondria (Takaya et al., 2003). Cytosolic GGPP synthases have so far only been found in non-plant eukaryotic systems (Ericsson et al., 1993; Hemmi et al., 2003). GGPP synthases from plants are structurally different from those of other eukaryotes (Hemmi et al., 2003).

The low overall rate of rubber biosynthesis in the presence of GGPP (Fig. 2) is not a reflection of low affinity of the *P. argentatum* rubber transferase for GGPP or for IPP in the presence of GGPP (Table 1); the transferase shows the highest substrate affinities in GGPP. The suppression of rate must reflect a partial inhibition of the IPP condensation reaction caused by the placement of the all *trans*-GGPP initiator in the active site although we have no information about the precise interference mechanism.

The kinetic properties of the *P. argentatum* rubber transferase appear to allow rubber production without adversely affecting plant growth and development. For example, rubber is largely synthesized during the winter, when the plant is essentially dormant and can afford to use large quantities of photo-assimilate on a compartmentalized secondary product, which it cannot recatabolize. During the summer, when *P. argentatum* is rapidly growing and is dependent upon the isoprenoid pathway to sustain the developmental processes (Fig. 1) the high rubber transferase K_m^{IPP}, which is at least 10-fold greater than known competing enzymes in the cytosol, ensures that rubber can only be made when IPP is not required for vital reactions. However, the K_m^{FPP} is smaller than the K_m^{FPP}s reported for other FPP-utilizing enzymes, indicating that rubber transferase can compete successfully for FPP in the presence of cytosolic

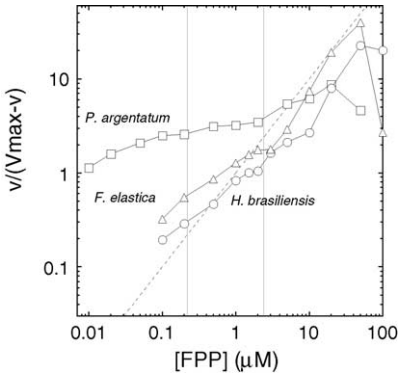

Fig. 8. Hill plot of IPP incorporation rate (5 mM IPP) as a function of FPP concentration for *P. argentatum* (□), *F. elastica* (△) and *H. brasiliensis* (◯). The vertical grey lines indicate the FPP concentrations selected to generate Fig. 10. (Data from Cornish et al., 2003.).

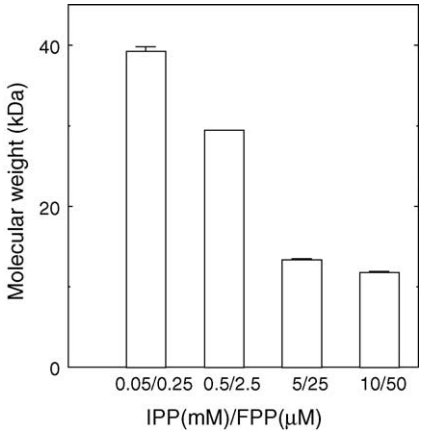

Fig. 9. Effect of increasing substrate concentration on the molecular weight of rubber molecules synthesized while maintaining a constant ratio of 200 IPP:1 FPP. Each value is the mean of three measurements.

FPP-requiring enzymes. Substrate deficits are avoided in this case because the FPP negativity cooperativity of the *P. argentatum* rubber transferase ensures that rubber biosynthesis does not deplete the FPP pool to the detriment of plant growth and development. It is noteworthy that the *F. elastica* and *H. brasiliensis* rubber transferases exhibit much less FPP negative cooperativity than the *P. argentatum* transferase (Fig. 8). This may reflect the disparate location of rubber production in the three species. *F. elastica* and *H. brasiliensis* both synthesize rubber in laticifers which are anatomically separate from the rest of the plant. Thus, production in laticifers depends on a flow of photo-assimilate to the laticifer, which then regulates the rate of rubber biosynthesis. When something goes amiss with regulation, rubber biosynthesis may cease but the tree continues to live (e.g., tapping panel dryness, Krishnakumar et al., 1999, 2001). In contrast, *P. argentatum* synthesizes its rubber in generalized bark parenchyma cells, which may make the process of rubber biosynthesis more dependent on developmental processes.

3.2. Molecular weight

It has previously been shown that rubber molecular weight in vitro is highly dependent upon the concentration and ratio of IPP and FPP (Cornish and Siler, 1995; Castillón and Cornish, 1999; Cornish et al., 2000). As substrate concentrations increase while maintaining a constant substrate ratio (Fig. 9), the rubber molecular weight produced by the *P. argentatum* rubber transferase decreases. Thus, FPP appears to exert the predominant regulatory effect and the higher the concentration of FPP, the lower the molecular weight.

Molecular weight increases with IPP concentration (Cornish and Siler, 1995; Castillón and Cornish, 1999; Cornish et al., 2000) and, as predicted, FPP negative cooperativity does appear to play a role in the regulation of molecular weight (Cornish et al., 2000). This may be illustrated by a comparison among the *P. argentatum*, *F. elastica* and *H. brasiliensis* rubber transferases (Fig. 10). In the presence of 0.25 μM FPP (Fig. 10A), molecular weight increases in all three species in an IPP-dependent manner, as non-limiting IPP concentrations continue to increase. This occurs in the *F. elastica* and *H. brasiliensis* rubber transferases because this FPP concentration is below their K_m^{FPP}s (Table 1) and so limits the chain-transfer reaction, the displacement of the elongating rubber molecule in the active site with a new initiator. Limiting the chain-transfer reaction allows polymer elongation to continue. However, 0.25 μM FPP is not a limiting initiator concentration for the *P. argentatum* rubber transferase, which has a much lower K_m^{FPP} (Table 1) than the other two species. Nonetheless, in this case, the IPP concentration dependent molecular weight increase is still mediated by inhibition of the chain-transfer reaction. At this FPP concentration, the first FPP bound to the enzyme, which initiates the polymerization reaction also impedes access of additional FPP molecules to the active site. This "negative cooperativity" occurs between between 0.1

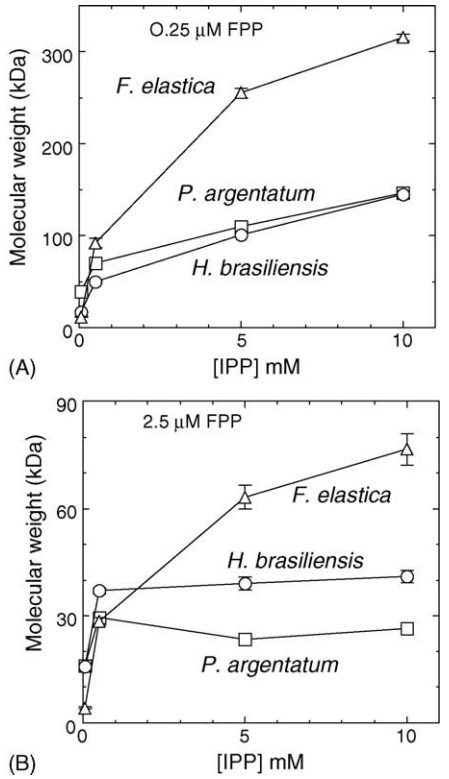

Fig. 10. Mean molecular weight ($\times 10^3$) of rubber molecules synthesized by *P. argentatum* (□), F. elastica (△) and *H. brasiliensis* (◯) as a function of IPP concentration and either (A) 0.25 μM FPP or (B) 2.5 μM FPP. Each value is the mean of 3. (Data from Cornish et al., 2003.).

and 2.0 μM FPP in the *P. argentatum* rubber transferase (Fig. 8).

In a test of this interpretation, the same experiment was repeated at a higher FPP concentration 2.5 μM (Fig. 10B). This concentration is above the K_m^{FPP} for all three rubber transferases (Table 1). It is also just outside the negative cooperativity concentration range for the *P. argentatum* rubber transferase. As expected, in the absence of substrate limitations or negative cooperativity, we found no IPP concentration dependent molecular weight increases above 500 μM IPP for either the *H. brasiliensis* or the *P. argentatum* rubber transferase. However, IPP concentration dependent molecular weight increases were apparent for *F. elastica* at this FPP concentration, again likely permitted by negative cooperativity for FPP, which appears to act on the *F. elastica* rubber transferase between 2.0 and 3.0 mM FPP (Fig. 8). Thus, in general, under non-limiting IPP and FPP concentrations, rubber molecular weights were independent of IPP concentration, except where the chain-transfer reaction was inhibited because of negative cooperativity. However, molecular weight regulation differs among the three rubber transferases because of their differences in intrinsic substrate affinities (K_ms) and the concentration range of initiating substrate at which the chain-transfer reaction is inhibited.

4. Conclusions

The *P. argentatum* rubber transferase has similar kinetic features as the other rubber producing species, such as high K_m^{IPP}. However, *P. argentatum* has evolved some unique rubber transferase features to regulate rubber biosynthesis, which enable it to synthesize high molecular weight rubber throughout seasonal changes in substrate availability and rubber transferase levels without injury to the plant itself.

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